Biochemistry (Moscow) 1999 vol.64 N8, pages 957-961

Preparation of Spin-Labeled Bacterial Ribonuclease from Bacillus intermedius 7P for EPR Studies of Protein Dynamics

Yagodina L., Altshuler A., Abreimova Y.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

The optimal conditions for labeling of binase (a bacterial ribonuclease isolated from Bacillus intermedius 7P) with a bromoacetamide spin label have been determined. The label is suitable for probing the dynamics of the protein by electron paramagnetic resonance (EPR). Binase samples specifically labeled at residue His-101 of the active center were prepared by incubation for 48 h at 20°C in potassium phosphate buffer (pH 5.5) containing the bromoacetamide (1:3 protein/label molar ratio). Fluorescence assay showed that the structure of the labeled binase is indistinguishable from that of the native protein.

Keywords

Binase, EPR, Fluorescence assay, Spin label